chain frameworks, which humanized immunoglobulin specifically binds to an antigen, wherein the sequence of the acceptor immunoglobulin heavy chain variable region framework is at least 65% identical to the sequence of the donor immunoglobulin heavy chain variable region framework, and the humanized immunoglobulin heavy chain variable region framework comprises at least 70 amino acids identical to those in the acceptor immunoglobulin heavy chain variable region framework, wherein percentage sequence identity is determined by aligning amino acids in said frameworks by Kabat numbering and wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin heavy chain framework outside the Kabat CDRs that replace the corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each of these said donor amino acids:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs.

(III)

30144. (New) A humanized immunoglobulin according to claim ~~143~~ 29, wherein the sequence of the acceptor immunoglobulin heavy chain variable region framework is at least 70% identical to the sequence of the donor immunoglobulin heavy chain variable region framework, wherein percentage sequence identity is determined by aligning amino acids in said frameworks by Kabat numbering.

Remarks

The courtesies extended by Examiners Reeves and Feisee at the recent interview with Applicants are appreciated. The suggested claims amendments have been introduced as discussed below. Claim amendments are for purposes of improved clarity or consistency of claim language unless otherwise noted. No claim amendment should be construed as an acquiescence in any ground of rejection.

Support for the recital of alignment of immunoglobulins by Kabat numbering is provided throughout the Specification, e.g., at Fig. 35, showing immunoglobulin sequences aligned by Kabat numbering, and by the statement at page 16, lines 29 to 32:

> Residues are numbered according to the Kabat system (E.A. Kabat et al., Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, MD) (1987).

The language of claims 112, 116 and 139-142 has also been modified slightly for better consistency with the language of claims 111 and 115.

Support for the recital of 65% or 70% identity between acceptor and donor variable region frameworks is provided, for example, at p. 30, lines 16-18 and p. 4, lines 17-20.

Support for replacing the phrase "Kabat and Chothia CDRs" with "hypervariable regions" in claims 113, 114, 119 and 120 is provided, for example, by the statement on page 63, lines 2 to 9:

> The chains all exhibit the same general structure of relatively conserved framework regions joined by **three hypervariable regions, also called Complementarity Determining Regions or CDR's** (see, "Sequences of Proteins of Immunological Interest," Kabat, E., et al., U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, J. Mol. Biol., 196, 901-917 (1987), which are incorporated herein by reference). (emphasis added)

Hence, hypervariable regions encompass both Kabat CDRs and Chothia CDRs so, e.g., an amino acid outside the hypervariable regions is outside both the Kabat and Chothia CDRs. Similarly, insertion of the clause "with the proviso that each of these said amino acids are outside Chothia CDR H1 (amino acids 26-32)" in claims 117, 118, 122, 123, 137 and 138 distinguishes these claims over and disclaims the teachings of Riechmann et al. (of record).

6. The title has been amended.

7. The cross-reference to related applications has been corrected.

8. A substitute specification will be provided on receipt of an indication of allowable subject matter.

9-10. A substitute declaration will be provided concurrent with a change of inventorship under separate cover in view.

11(a). Claims 114, 118, 120-121, 124-126, 131-135, 137, and 142 stand rejected under 35 USC 112, second paragraph on the ground that the claims are indefinite for failure to recite that claimed antibodies have a binding affinity within fourfold of the donor.

G

This rejection appears to raise the same underlying issue as the related rejection under 35 USC 112, first paragraph, and is addressed below in the discussion regarding Section 15.

13-14. Claims 111-112, 115, 116, and 139-142 stand rejected under 35 USC 112, first and second paragraphs on the ground that the Specification does not enable one to produce a variable region framework which is at least 65% or 70% identical to the donor variable region framework. The Examiner writes that predictability of which changes can be tolerated in a protein's amino acid sequence, while still obtaining the desired activity of the protein requires guidance as to which amino acids in the protein's sequence are tolerant of modification, and which are conserved. The Examiner also indicates that sequence identity between two sequences may have no common meaning in the art in that the scoring of gaps when comparing one sequence to another introduces uncertainty as to the percent of similarity between two sequences. This rejection is respectfully traversed as it applies to the present claims.

The Office Action's first issue appears to be that undue experimentation may be required for humanized immunoglobulins showing only 65% or 70% sequence identity to a donor immunoglobulin to retain similar binding characteristics to the donor. However, the premise on which this concern is based, namely, that Applicants allegedly had not identified which amino acids are tolerant of modification and which are conserved, is incorrect. The conserved residues are the donor CDRs and certain framework amino acids outside the Kabat and Chothia CDRs (the framework region is defined on, e.g., page 64, lines 3 to 7). The subject Specification provides criteria (e.g., categories 1-4 on pages 31 to 32) for recognizing these amino acids, which should be conserved, and exemplifies the application of these criteria in the successful humanization of several mouse antibodies. Moreover, the remaining framework residues, which are relatively tolerant of change, are not selected at random but rather are typically obtained entirely or substantially from the variable framework region of a human acceptor antibody. Thus, by following the teaching of the Specification to select donor amino acids at CDRs and certain critical positions, and selecting noncritical positions from the variable framework region of a human antibody, antibodies having a sequence identity of 65% with the donor and having similar binding characteristics as the donor are produced.

G

With respect to the issue of percent sequence identity, the Office Action alleges that it is unclear which algorithm and what parameters are being used to calculate percent sequence identity. This appears to be an issue of claim definiteness.

The claims have been amended to state that sequence identity is determined by aligning the framework sequences being compared by Kabat numbering. Such is in accordance with all the examples of aligned framework sequences in the subject Specification, such as Fig. 1-6, but is perhaps illustrated most clearly in Fig. 35A-D. The widely accepted Kabat numbering provides a unique method of aligning any two immunoglobulin sequences, which in turn provides a well-defined meaning for percentage sequence identity of antibody frameworks. Specifically, in the Kabat system, amino acids from the variable regions of the mature heavy and light chains of immunoglobulins are denoted Hx and Lx respectively, where x is the number of the designated amino acid position (see Kabat et al., Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services, 1987 and 1991). Two immunoglobulin sequences are aligned by aligning each of the respective amino acids with the same number, for example, amino acid H50 of a human antibody would be aligned with amino acid H50 of a mouse antibody. Using this method, Kabat et al. (*ibid.*) successfully and uniquely aligned some hundreds of mouse and human immunoglobulin sequences. An example of alignment of a large number of immunoglobulin sequences by Kabat numbering is provided in Exhibit 2 of the Declaration of Dr. Cary Queen submitted with this Amendment. In his Declaration, Dr. Queen indicates that the Kabat numbering system and Kabat's alignments of immunoglobulin sequences were so well known at the filing dates of the Specification (and still are today), that anyone of skill in the art would have aligned immunoglobulin framework sequences in this way.

Dr. Queen further explains that the success of the Kabat numbering system, the ability to number any immunoglobulin sequence according to this system, and the resulting ability to align antibody frameworks by Kabat numbering, is fundamentally due to the almost complete absence of gaps in framework sequences (with respect to other framework sequences of the same type of chain). This is illustrated in Exhibit 2, a representative page of Kabat's compendium, where 22 human immunoglobulin sequences are aligned without a single gap in the frameworks, and also in Exhibit 3, where 83 human immunoglobulin heavy chain germline genes are aligned, again without a single framework gap. Hence, the Examiner's comment that

G

"The scoring of gaps ... introduces uncertainty," while possibly correct for some proteins, is simply not germane to immunoglobulin framework sequences. Scoring of gaps is irrelevant when there are no gaps. Finally, Dr. Queen notes that when there are no gaps, there is only one sensible way to number and align two related sequences, and the Kabat numbering system is essentially a reflection of that fact for immunoglobulin framework sequences.

To further underscore that immunoglobulin framework sequences are aligned in only one way, the Applicant has also submitted with this Amendment a Declaration by Dr. Maximiliano Vasquez, an expert in antibody structure and humanization. Dr. Vasquez has used the GAP program of the widely used Wisconsin Package for sequence analysis (Genetics Computer Group, Madison, WI) to align two relevant and representative heavy chain framework sequences, namely the mouse anti-Tac sequence and the human EU sequence, which are respectively the donor and acceptor immunoglobulin in the first experimental example of the subject Specification (pages 75 to 82 and Figure 1). The program provides the flexibility to align any two protein sequences using many different similarity matrices and gap penalties. Dr. Vasquez instructed the program to align the two framework sequences using seven different similarity matrices and ten different gap penalties, thus using a wide variety of alignment algorithms, including several particularly recommended by Barton et al. (cited in the Office Action) and by the program itself. In every case, as seen in Exhibit 3 of the Vasquez Declaration, the alignment was the same, and so was the percent identity (67%). This unique alignment generated by all the methods was the same as the alignment by Kabat numbering and had no gaps. Hence, while the Examiner may be correct that for some protein sequences the scoring of gaps introduces uncertainty, Dr. Vasquez' Declaration shows clearly that this is not the case for immunoglobulin framework sequences, since the program with any gap penalty generated precisely the same alignment and percent identity as did Kabat numbering. Finally, Dr. Vasquez added that alignment of framework sequences by Kabat numbering is known to produce the closest physical alignment of the corresponding 3-dimensional crystal structures. Therefore, even if other sequence alignments are possible, one of skill would reject them as not being biologically relevant.

Lastly, it should be noted that once two framework sequences are aligned, the phrases "percent identical" and "percent sequence identity" or "percentage identity" have a well-defined meaning. That is simply the percentage of the aligned units (in this case amino

acids) that are respectively identical (i.e., are the same). So, for example, if 87 framework amino acids are aligned, and 58 of them match, as in Exhibit 3 of the Vasquez Declaration, the percent identity is 100 x (58/87) = 67%. No other meaning is consistent with the mathematical definition of "percent" or with plain English. Of course, other definitions of similarity are possible, and these are frequently referred to as a similarity score or sometimes an identity score, since certainly a score may be calculated in many ways. For example, Barton (of record) lists a number of such scoring schemes (page 31 to 35). However, Barton also states on page 42:

> Percentage identity is **a** frequently quoted statistic for an alignment of two sequences. (emphasis added)

Thus, Barton agrees that percentage identity is one particular statistic (calculated value) that has a unique meaning once two sequences are aligned. In addition, in his Declaration, Dr. Vasquez notes that the widely used Wisconsin software package also interprets "percent identity" in the usual way, stating specifically: "Percent Identity is the percent of symbols that actually match."

For these reasons, withdrawal of the rejection is respectfully requested.

15. Claims 114, 118, 120-121, 123, 124-125, 126, 131, 132-137, 142 stand rejected under 35 USC 112, first paragraph. The Examiner acknowledges that the methods of humanizing antibodies disclosed in the subject Specification have achieved increases of affinity of the order of 3-8 fold relative to the donor antibody, but alleges that the claims read on antibodies having much stronger affinities than their donor antibody and should be limited to recite an upper limit of within four-fold of the donor antibody. This rejection is respectfully traversed as it applies to the pending claims, which do not recite a particular affinity that must be obtained. Thus, the question of whether the Specification generally enables increasing the affinity of an antibody by humanization, e.g., from a low affinity of 10^5 M^{-1} to a moderate affinity of 10^8 M^{-1}, or from a moderate affinity of 10^8 M^{-1} to a high affinity of 10^{11} M^{-1}, is not relevant to these claims, for the reasons detailed below.

C

Although the Office Action acknowledges that the methods disclosed by the present Specification can lead to humanized antibodies having at least eight-fold greater affinity than the donor, the Office Action appears to assume that there exists a class of humanized antibodies having still greater affinity that could be made by some other unspecified method, but not by the methods disclosed in the Specification. Applicants disagree that such a class of humanized antibodies is reasonably expected to exist. Rather, it is Applicants' position that the extent of affinities attainable in humanized antibodies using the methods disclosed in the present Specification is substantially coextensive with affinities that inherently reside in humanized antibodies encompassed by the claims at issue. Thus, the enablement provided by the Specification is commensurate with the scope of the claims.

The Office Action's assumption that there exists a class of humanized antibodies having significantly higher affinity than that attainable by the methods disclosed in the present Specification appears to arise due to Grove's reported isolation of a sheep monoclonal antibody having an affinity of the order of 10^{11} M^{-1}. Presumably, the Office Action's rationale is that if one can make a sheep antibody with an affinity of 10^{11} M^{-1}, then it should be possible to make any humanized antibody having the same affinity. This rationale is mistaken. The high affinity of the sheep antibody reported by Grove presumably resides in the residues that principally effect binding, namely, the CDR region and a few variable region frameworks amino acids that interact with the CDRs. Although it should be possible to humanize Grove's particular antibody and achieve an affinity of 10^{11} M^{-1}, this does not mean that donor antibodies in general can be humanized with such affinities. A humanized antibody incorporates CDR regions and key framework residues from the particular donor antibody being humanized, and thereby precludes incorporation of the same residues from a different donor, such as Grove's sheep antibody.

For these reasons, it is reasonably expected that such inherent features of a donor as may limit the affinities of humanized antibodies produced by the present methods, apply equally to other methods, and that the enablement provided by the Specification is thus commensurate with claim scope. In these circumstances, it is unnecessary and unreasonable to expect Applicants to identify a precise point of demarcation between feasible and infeasible affinities and expressly recite this in the claims so as to exclude infeasible affinities. In general, the claims serve to define what an invention is, rather than what an invention is not.

For any claimed invention, one can identify an infinite number of theoretical embodiments that in all likelihood could never be made in practice. For example in the present case, one could theoretically imagine humanized antibodies that are resistant to autoclaving or which simultaneously cure two unrelated diseases, but it would almost certainly be impossible to make any such antibody. To expect Applicants to identify all such theoretically imaginable but practically impossible embodiments at the time of filing, and expressly exclude them from the claims would be an impossible and unreasonable burden, and is not the law.

It is perhaps for this reason that numerous US patents have issued which claim compositions and methods of humanizing or otherwise engineering antibodies while maintaining their affinity, without any explicit exclusion of antibodies exceeding an upper limit of affinity (see, e.g., US 5,766,886, US 5,225,539, US 5,639,641 and US 5,859,205). In each case, it is clear that some upper limit must exist. Nevertheless, it would have been an unreasonable and unnecessary burden to expect the patentees to have defined precisely what the limit was, and such was not required.

For these reasons, it is submitted that the present claims need not recite an upper limit of binding affinity, and that the rejection should be withdrawn.

18. Claims 111 and 115 stand rejected as anticipated by Riechmann as evidenced by Cheetham. The Office Action refers to Applicants' arguments to the effect that Riechmann does not disclose substitutions outside Chothia CDR H1, but says that such arguments are not applicable to claims 111 and 115, because these claims do not recite this limitation. This rejection is respectfully traversed.

With respect, the Office Action seems to be overlooking a separate ground of patentability for claims 111 and 115 . Namely, these claims specify steps of comparing the sequence of a donor immunoglobulin heavy chain variable region against a collection of sequences of human heavy chain variable regions, and of selecting a human heavy chain variable region from the collection of human heavy chain variable regions to provide an acceptor heavy chain variable region, wherein the selected variable region framework is at least 65% identical to the donor immunoglobulin heavy chain variable region framework.

The present claims are distinguished over Riechmann in at least two respects. First, the donor and acceptor antibodies of Riechmann do not show 65% identity between their

G

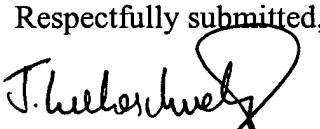
heavy chain variable region frameworks, as recited in the noted claims. This can be seen by referring to corrected Appendix 1, which shows that the identity was only 53%. Second, the NEW antibody used by Riechmann as human acceptor heavy chain framework, was not selected as a result of comparing the sequence of Riechmann donor antibody with a collection of human antibody heavy chain sequences, but rather because of the availability of an X-ray structure for the NEW antibody (see Jones et al. (of record), page 523, lines 6-9). Further, nothing in Riechmann suggested that the extent of identity between the donor and acceptor or humanized heavy chain was in any way relevant to successful humanization. Thus, Riechmann did not provide motivation either to compare the sequence of a donor antibody heavy chain with that of a collection of human antibodies or to select a human antibody heavy chain having at least 65% sequence identity to the donor.

New claim 143 (and new claim 144) are similarly distinguished over Riechmann because Riechmann's humanized antibody and its mouse donor antibody do not show at least 65% (or 70%) identity between their heavy chain variable region frameworks, as recited in claims 143 and 144 respectively.

Therefore, none of the claims was anticipated or rendered obvious by Riechmann.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400.

Respectfully submitted,



Joe Liebeschuetz
Reg. No. 37,505

William M. Smith
Reg. No. 30,223

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
JOL
PA 3007376 v2

G

APPENDIX 1

(DONOR) RAT CAMPATH-1 HEAVY CHAIN
(ACCEPTOR) HUMAN NEW HEAVY CHAIN

																			Match	All
E	V	K	L	L	E	S	G	G	G	L	V	Q	P	G	G	S	M	R	L	
	|		|		|	|	|		|	|	|		|						|	10	20
Q	V	Q	L	Q	E	S	G	P	G	L	V	R	P	S	Q	T	L	S	L	
S	C	A	G	S	G	F	T	F	T	D	F	Y	M	N	W	I	R	Q	P	
	|			|	|		|	|				|		|		|		|		|	|	|	9	15
T	C	T	V	S	G	S	T	F	S	N	D	Y	Y	T	W	V	R	Q	P	
A	G	K	A	P	E	W	L	G	F	I	R	D	K	A	K	G	Y	T	T	
	|				|	|		|											|	4	9
P	G	R	G	L	E	W	I	G	Y	V	F	Y	H	G	T	S	D	D	T	
E	Y	N	P	S	V	K	G	R	F	T	I	S	R	D	N	T	Q	N	M	
				|				|		|				|				|			4	12
T	P	L	R	S				R	V	T	M	L	V	D	T	S	K	N	Q	
L	V	L	Q	M	N	T	L	R	A	E	D	T	A	T	Y	Y	C	A	R	
		|							|		|	|	|		|	|	|	|	|			10	20
F	S	L	R	L	S	S	V	T	A	A	D	T	A	V	Y	Y	C	A	R	
E	G	H	T	A	A	P	F	D	Y	W	G	Q	G	V	M	V	T	V	S	
				|				|		|	|	|	|			|	|	|	|			8	10
	N	L	I	A	G	C	I	D	V	W	G	Q	G	S	L	V	T	V	S	
S																				
|																			1	1
S																				
																			46	87

Percent Identity = 46/87 = 53%

Legend. The donor and acceptor antibodies of Riechmann et al. are aligned, with vertical lines indicating identity of amino acids. The Kabat CDRs are underlined. The number of framework amino acids on each line and the number that match (i.e., are identical) are listed next to each line. The total and number of matching framework amino acids, and percent identity, are shown.

CARY L. QUEEN

Protein Design Labs, Inc.
2375 Garcia Avenue
Mountain View, CA 94043
415-903-3700

EDUCATION:

1971-1975	UNIVERSITY OF CALIFORNIA, Berkeley, CA Ph.D., Mathematics Thesis Advisor: Dr. Andrew Ogg Thesis Topic: p-adic L-functions
1968-1971	COLUMBIA UNIVERSITY, New York, NY B.A. Mathematics summa cum laude

PROFESSIONAL EXPERIENCE:

1993- present	PROTEIN DESIGN LABS, INC., Mountain View, CA Senior Vice President and Vice President, Research
1989-1993	PROTEIN DESIGN LABS, INC., Palo Alto, CA Vice President, Research
1987-1988	PROTEIN DESIGN LABS, INC., Palo Alto, CA Scientific Director
1982-1986	NATIONAL CANCER INSTITUTE, Bethesda, MD Independent Investigator, Laboratory of Biochemistry Research area: Immunoglobulin gene expression
1981-1982	MASSACHUSETTS INSTITUTE OF TECHNOLOGY, Cambridge, MA NIH Postdoctoral Fellow, Center for Cancer Research Research Advisor: Dr. David Baltimore Research Area: Immunoglobulin gene expression
1978-1981	NATIONAL CANCER INSTITUTE, Bethesda, MD Staff Fellow, Laboratory of Biochemistry Research Advisors: Dr. Maxine Singer & Dr. Martin Rosenberg Research Areas: Regulation of transcription and translation
1975-1978	CORNELL UNIVERISTY, Ithaca, NY Assistant Professor, Department of Mathematics Research Areas: Algebraic Number Theory, Computer Analysis of DNA
1971-1975	UNIVERSITY OF CALIFORNIA, Berkeley Woodrow Wilson and National Science Foundation Pre-doctoral Fellow, Department of Mathematics Research Area: Algebraic Number Theory

PUBLICATIONS:

1. Queen, C. (1977). The existence of p-adic abelian L-functions. In Number Theory and Algebra, R. Bejunic, Ed., Academic Press, New York, pp. 263-287.

2. Korn, L., Queen, C., and Wegman, M. (1977). Computer analysis of nucleic acid regulatory sequences. *Proc. Natl. Acad. Sci.* USA **74**: 4401-4405.

3. Queen, C. and Korn, L. (1980). Computer analysis of nucleic acids and proteins. *Methods Enzymol.* **65**: 595-609.

4. Sege, R., Soll, D., Ruddle, F., and Queen, C. (1981). A conversational system for the computer analysis of nucleic acid sequences. *Nucleic Acids Res.* **9**: 437-444.

5. Queen, C. and Rosenberg, M. (1981). Differential translation efficiency explains discoordinate expression of the galactose operon. *Cell* **25**: 241-249.

6. Queen, C. and Rosenberg, M. (1981). A promoter on pBR322 activated by cAMP receptor protein. *Nucleic Acids Res.* **9**: 3365-3377.

7. Queen, C., Lord, S.T., McCutchan, T.F., and Singer, M.F. (1981). Three segments from the monkey genome that hybridize to SV40 have common structural elements. *Mol. Cell. Biol.* **1**: 1061-1068.

8. Grimaldi, G., Queen, C., and Singer, M.F. (1981). Interspersed repeated sequences in the African green monkey genome that are homologous to the human Alu family. *Nucleic Acids Res.* **9**: 5553-5568.

9. Queen, C., Wegman, M., and Korn, L.J. (1982). Improvements to a program for DNA analaysis: a procedure to find homologies among many sequences. *Nucleic Acids Res.* **10**: 449-456.

10. Wang, J., Queen, C., and Baltimore, D. (1982). Expression of an Abelson murine leukemia virus-encoded protein in Escherichia coli causes extensive phosphorylation of tyrosine residues. *J. Biol. Chem.* **257**: 13181-13184.

11. Queen, C. (1983). A vector that uses phage signals for efficient synthesis of proteins in Escherichia coli. *J. Mol. Applied Gen.* **2**: 1-10.

12. Queen, C. and Baltimore, D. (1983). Immunoglobulin gene transcription is activated by downstream sequence elements. *Cell* **323**: 741-748.

13. Stafford, J. and Queen, C. (1983). Cell-type specific expression of a transfected immunoglobulin gene. *Nature* **306**: 77-79.

14. Queen, C. and Korn, L.J. (1984). A comprehensive sequence analysis program for the IBM personal computer. *Nucleic Acids Res.* **12**: 581-599.

15. Queen, C. and Stafford, J. (1984). Fine mapping of an immunoglobin gene activator. *Mol. Cell. Biol.* **4**: 1042-1049.

16. Queen, C. (1984). Regulation of immunoglobin transcription. *Oxford Surveys on Eucaryotic Genes* **1**: 169-191.

17. Korn, L.J. and Queen, C. (1985). Analysis of biological sequences on small computers. *DNA* **3**: 421-436.

18. Foster, J., Stafford, J., and Queen, C. (1985). An immunoglobin gene promoter displays cell-type specificity independently of the enhancer. *Nature* **315**: 423-425.

19. Queen, C., Foster, J., Stauber, C., and Stafford, J. (1986). Cell-type specific regulation of an immunoglobin gene by promoter and enhancer elements. *Immunol. Rev.* **89**: 49-68.

20. Bich-Thuy, L.T., Queen, C., and Fauci, A.S. (1986). Interferon gamma induces light chain synthesis in interleukin-2 stimulated human B cells. *Eur. J. Immunol.* **16**: 547-550.

21. Garcia, J.V., Stafford, J., and Queen, C. (1986). Synergism between a light chain enhancer and promoter. *Nature* **322**: 383-385.

22. Steege, D.A., Cone, K.C., Queen, C., and Rosenberg, M. (1987). Bacteriophage Lambda N gene leader RNA. *J. Biol. Chem.* **262**: 17651-17658.

23. Bich-Thuy, L.T. and Queen, C. (1988). Transfection of an immunoglobulin κ gene into mature human B lymphocytes. *Mol. Cell. Biol.* **8**: 511-513.

24. Chaudhary, V.K., Queen, C., Junghans, R.P., Waldmann, T.A., FitzGerald, D., and Pastan, I. (1989). A recombinant immunotoxin consisting of two antibody variable domains fused to Pseudomonas exotoxin. *Nature* **339**: 394-397.

25. Bich-Thuy, L.T. and Queen, C. (1989). An enhancer associated with the mouse lambda immunoglobulin gene is specific for lambda light chain producing cells. *Nucleic Acids Res.* **17**: 5307-5321.

26. Queen, C., Schneider, W.P., Selick, H.E., Payne, P.W., Landolfi, N.F., Duncan, J.F., Avdalovic, N.M., Levitt, M., Junghans, R.P., and Waldmann, T.A. (1989). A humanized antibody that binds to the IL-2 receptor. *Proc. Natl. Acad. Sci. USA* **86**: 10029-10033.

27. Junghans, R.P., Waldmann, T.A., Landolfi, N.F., Avdalovic, N.M., Schneider, W.P., and Queen, C. (1990). Anti-Tac-H, a humanized antibody to the interleukin 2 receptor with new features for immunotherapy in malignant and immune disorders. *Cancer Res.* **50**: 1495-1502.

28. Chovnick, A., Schneider, W.P., Tso, J.Y., Queen, C., and Chang, C.N. (1991). A recombinant, membrane-acting immunotoxin. *Cancer Research* **51**: 465-467.

29. Co, M.S., Deschamps, M., Whitley, R.J., and Queen, C. (1991). Humanized antibodies for antiviral therapy. *Proc. Natl. Acad. Sci. USA* **88**: 2869-2873.

30. Brown, Jr., P.S., Parenteau, G.L., Dirbas, F.M., Garsia, R.J., Goldman, C.K., Bukowski, M.A., Junghans, R.P., Queen, C., Hakimi, J., Benjamin, W.R., Clark, R.E., and Waldmann, T.A. (1991). Anti-Tac-H, a humanized antibody to the interleukin-2 receptor prolongs primate cardiac allograft survival. *Proc. Natl. Acad. Sci. USA* **88**: 2663-2667.

31. Co, M.S. and Queen, C. (1991). Humanized antibodies for therapy. *Nature* **351**: 501-502.

32. Hakimi, J., Chizzonite, R., Luke, D.R., Familletti, P.C., Bailon, P., Kondas, J.A., Pilson, R.S., Lin, P., Weber, D.V., Spence, C., Mondini, L.J., Tsien, W.-H., Levin, J.L., Gallati, V.H., Korn, L., Waldmann,T.A., Queen, C. and Benjamin, W.R. (1991). Reduced immunogenicity and

improved pharmacokinetics of humanized anti-Tac in cynomolgus monkeys. *J. Immunol.* **147**: 1352-1359.

33. Chang, C.N., Landolfi, N.F. and Queen, C. (1991). Expression of antibody Fab domains on bacteriophage surfaces. *J. Immunol.* **147**: 3610-3614.

34. Co, M.S., Avdalovic, N.M., Caron, P.C., Avdalovic, M.V., Scheinberg, D.A. and Queen, C. (1992). Chimeric and humanized antibodies with specificity for the CD33 antigen. *J. Immunol.* **148**: 1149-1154.

35. Kreitman, R.J., Schneider, W.P., Queen, C., Tsudo, M., Fitzgerald, D.J.P., Waldmann, T. A., and Pastan, I. (1992). Mik-ß1(Fv)-PE40, A recombinant immunotoxin cytotoxic toward cells bearing the ß-chain of the IL-2 receptor. *J. Immunol.* **149**: 2810-2815.

36. Caron, P.C., Laird, W., Co, M.S., Avdalovic, M.N., Queen, C. and Scheinberg, D.A. (1992). Engineered humanized dimeric forms of IgG are more effective antibodies. *J. Exp. Med.* **176**: 1191-1195.

37. Caron, P.C., Co, M.S., Bull, M.K., Avdalovic, N.M., Queen, C. and Scheinberg, D.A. (1992). Biological and immunological features of humanized M195 (anti-CD33) monoclonal antibodies. *Cancer Res.* **52**: 6761-6767.

38. Co, M.S., Scheinberg, D.A., Avdalovic, N.M., McGraw, K., Vasquez, M., Caron, P.C., and Queen, C. (1993). Genetically engineered deglycosylation of the variable domain increases the affinity of an anti-CD33 monoclonal antibody. *Mol. Immunol.* **30**: 1361-1367.

39. Queen, C., Schneider, W.P., and Waldmann, T.A. (1993). Humanized antibodies to the IL-2 receptor. In Protein Engineering of Antibody Molecules for Prophylactic and Therapeutic Applications in Man, Clark, M., ed. (Nottingham, England: Academic Titles), pp. 159-170.

40. Co, M.S., Yano, S., Hsu, R., Landolfi, N.F., Vasquez, M., Cole, M., Tso, J.Y., Bringman, T., Laird, W., Hudson, D., Kawamura, K., Suzuki, K., Furuchi, K., Queen, C., and Masuho, Y. (1994). A humanized antibody specific for the platelet integrin gpIIb/IIIa. *J. Immunol.* **152**: 2968-2976.

41. Caron, P.C, Schwartz, M.A., Co, M.S., Queen, C., Finn, R.D., Graham, M.C. and Scheinberg, D.A. (1994). Murine and humanized constructs of monoclonal antibody M195 (anti-CD33) for the therapy of acute myelogenous lukemia. *Cancer* **73**: 1049-1056.

42. Kreitman, R.J., Chang, C.N., Hudson, D.V., Queen, C., Balion, P, and Pastan, I. (1994). Anti-Tac(Fab)-PE40, A recombinant double-chain immunotoxin which kills interleukin-2-receptor-bearing cells and includes complete remission in an *in vivo* tumor model. *Int. J. Cancer* **57**: 856-864.

43. Buerke, M., Weyrich, A.S., Murohara, T., Queen, C., Klingbeil, C.K., Co, M.S., and Lefer, A.M. (1994). Humanized mAb DREG-200 directed against L-selectin protects in feline myocardial reperfusion injury. *J. Pharmacol. Exper. Therap.* **271**: 134-142.

44. Östberg, L. and Queen, C. (1995). Human and humanized monoclonal antibodies: Preclinical studies and clinical experience. *Therapeutic Monoclonals* **23**: 1038-1043.

45. Co, M.S., Baker, J., Bednarik, K., Janzek, E., Neruda, W., Mayer, P., Plot, R., Stumper, B., and Vásquez, M., Queen C., and Loibner, H. (1996). Humanized anti-Lewis Y antibodies: in vitro properties and pharmacokinetics in Rhesus monkeys. *Cancer Res.* **56**: 1118-1125.

Table. Amino acid substitutions in Adair et al. patent application WO 91/09967 made according to criteria in specification of Queen et al., U.S. Patent No. 5,585,089.

Substitution in OKT3 by Adair et al. (WO 91/09967)	Meets "Queen" adjacent criterion (i)	Meets "Queen" interaction criterion (and within 6 Å) (ii)	Meets "Queen" rareness criterion (iii)
Light chain			
1	-	Yes	-
3	-	Yes	-
46	-	Yes	-
47	-	Yes	-
Heavy chain			
6	-	-	-
23	-	Yes	-
24	-	Yes	Yes
48	-	Yes	-
49	Yes	Yes	-
71	-	Yes	-
73	-	Yes	-
76	-	Yes	-
78	-	Yes	-
88	-	-	Yes
91	-	-	Yes

Figure 1A

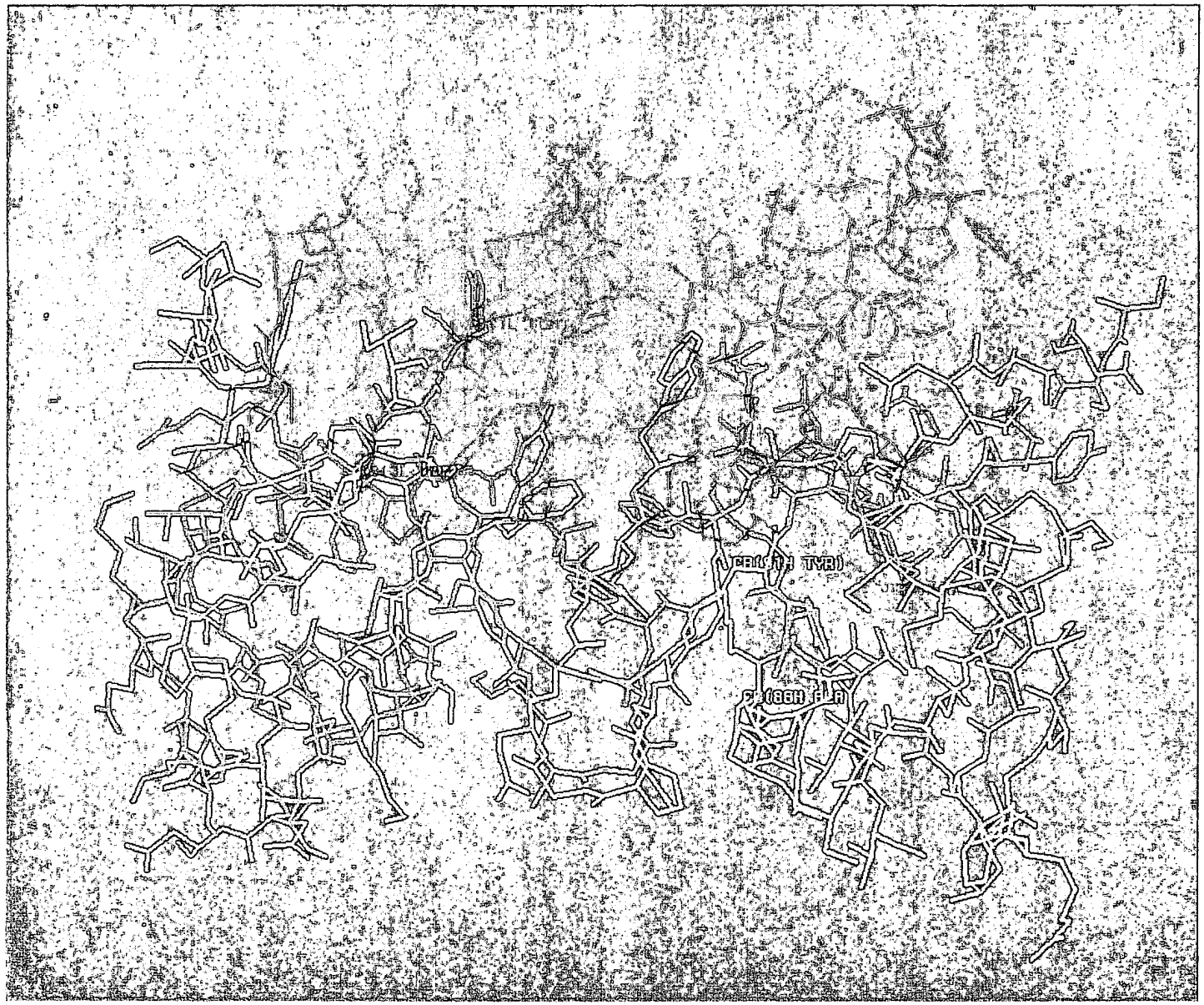


Figure 2A

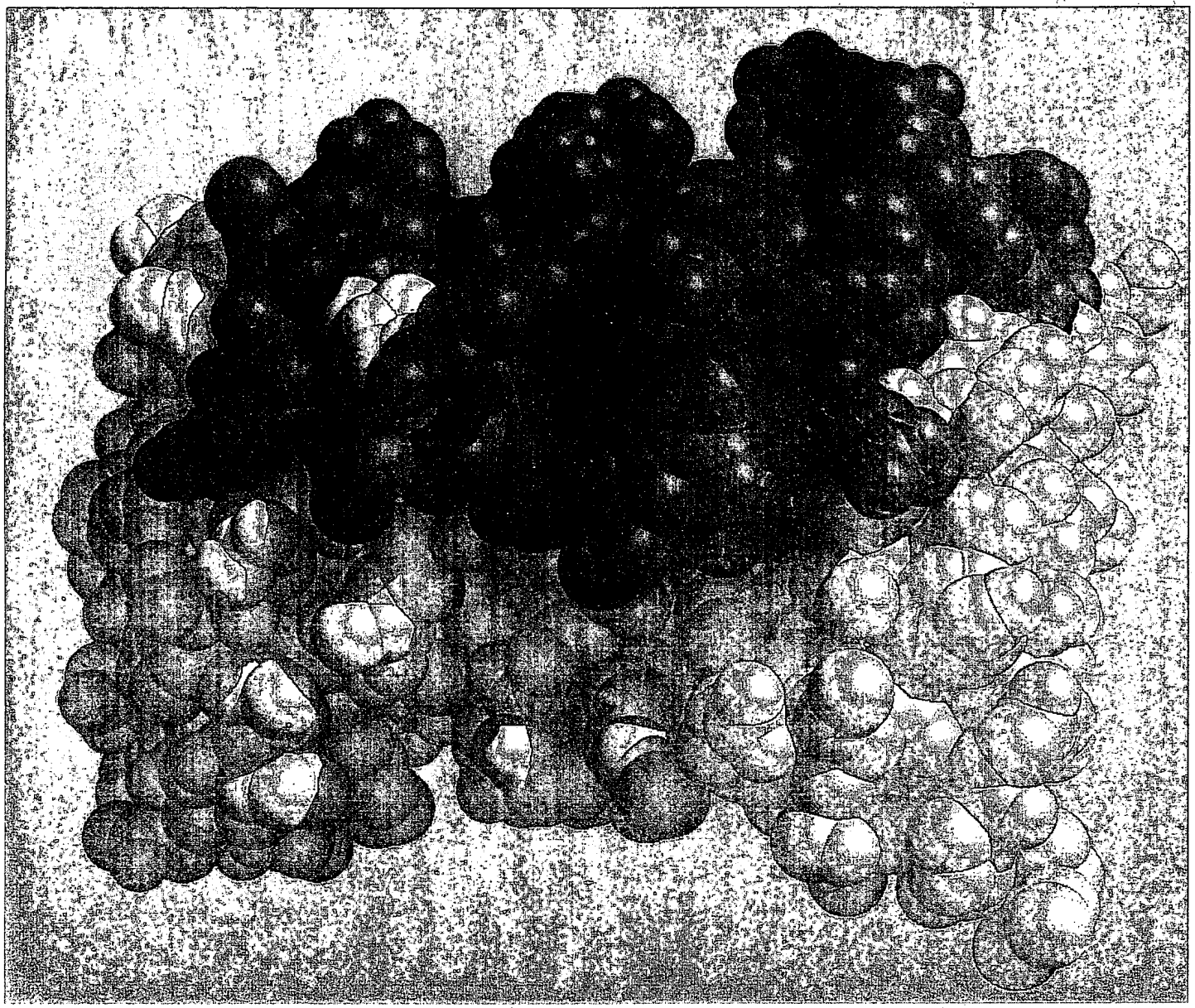


Figure 1B

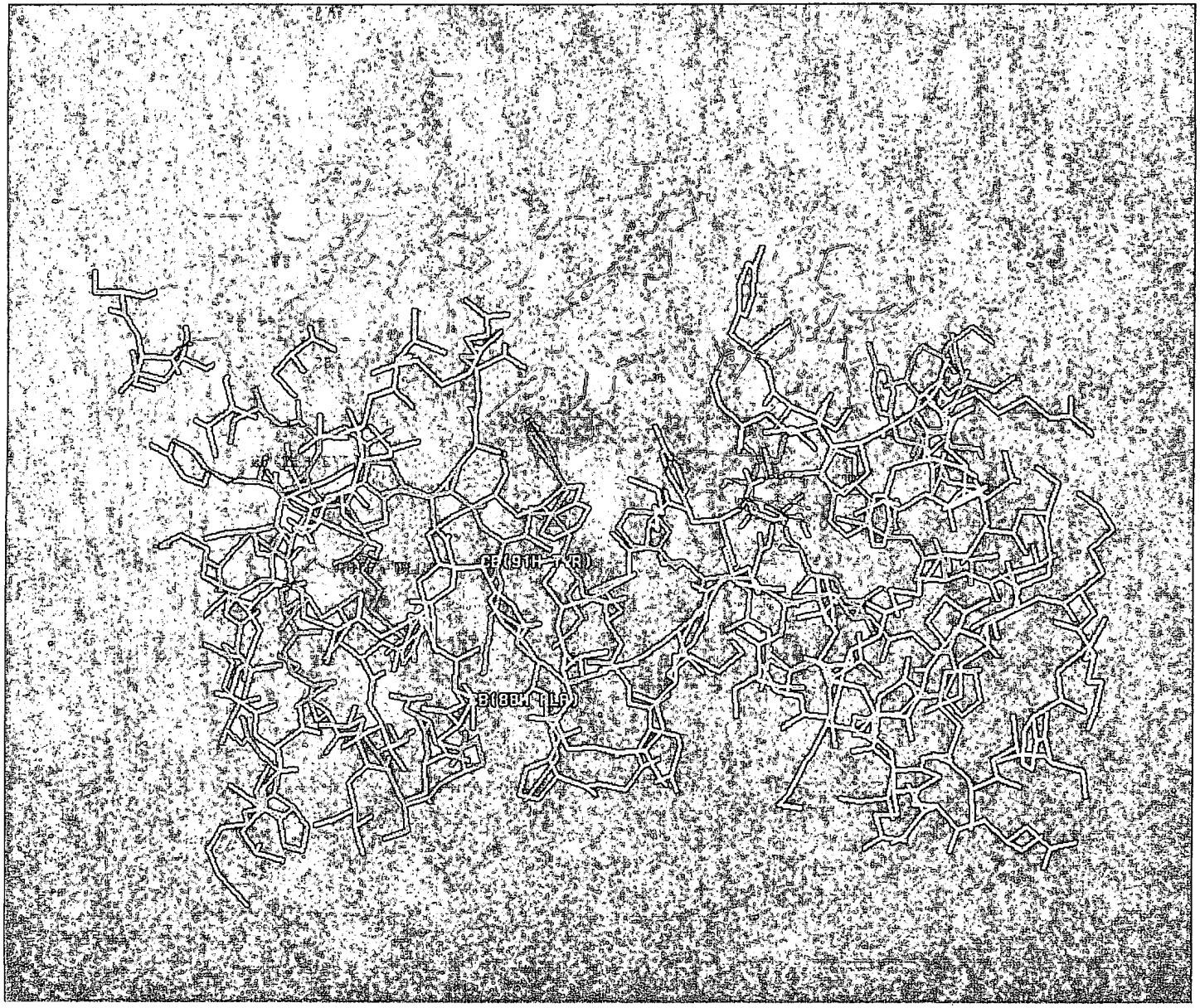


Figure 2B

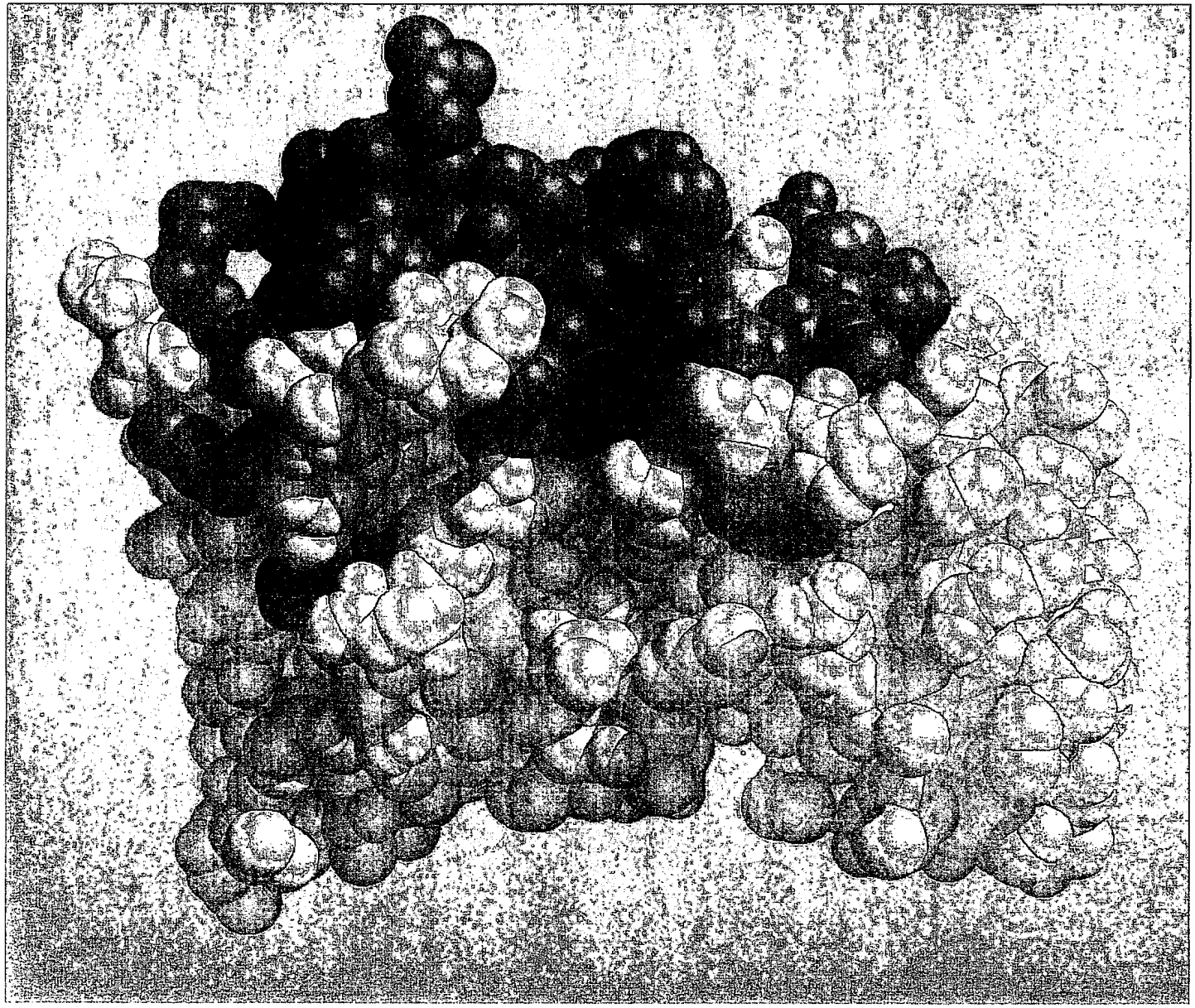


Figure 1A

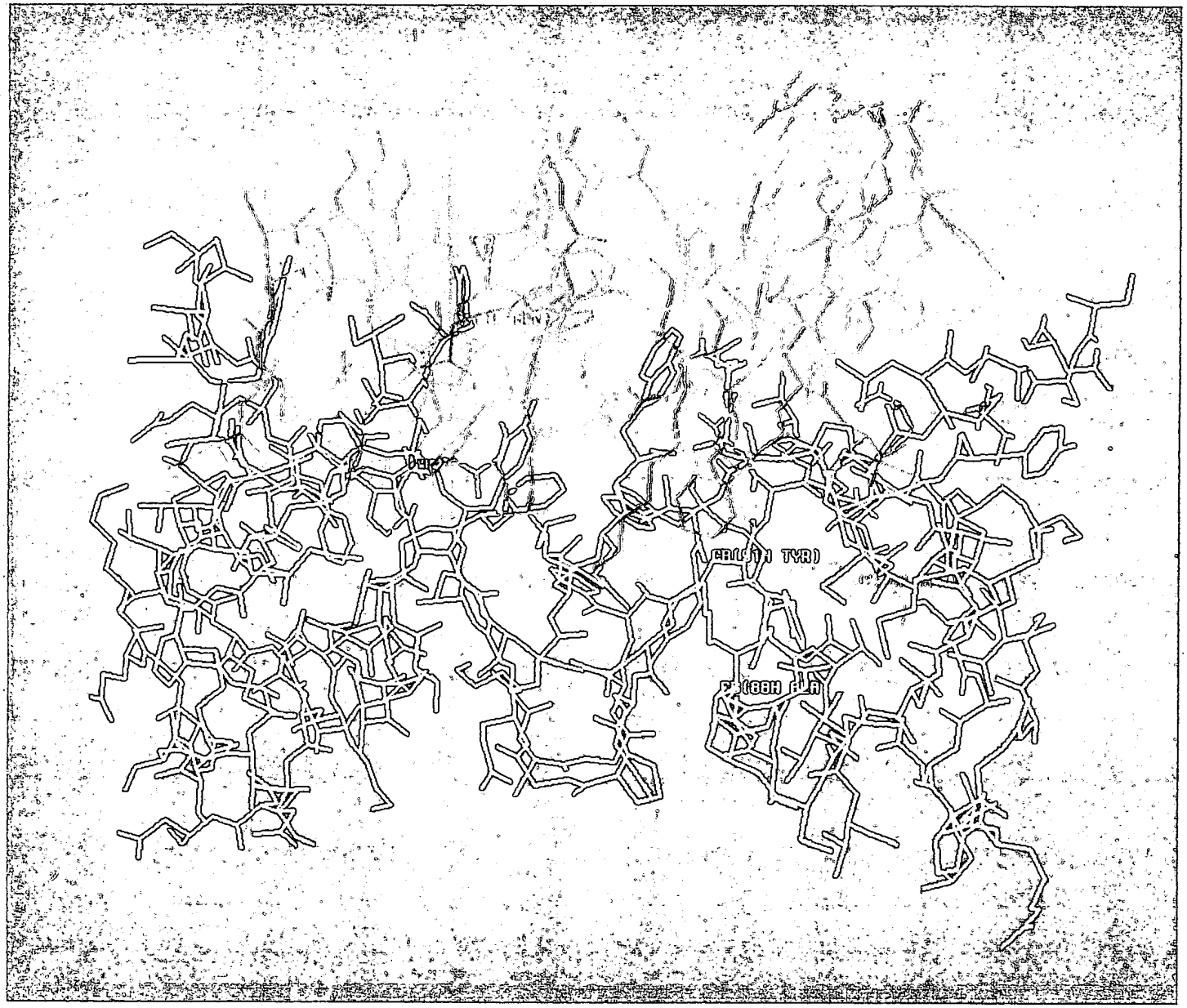


Figure 1B

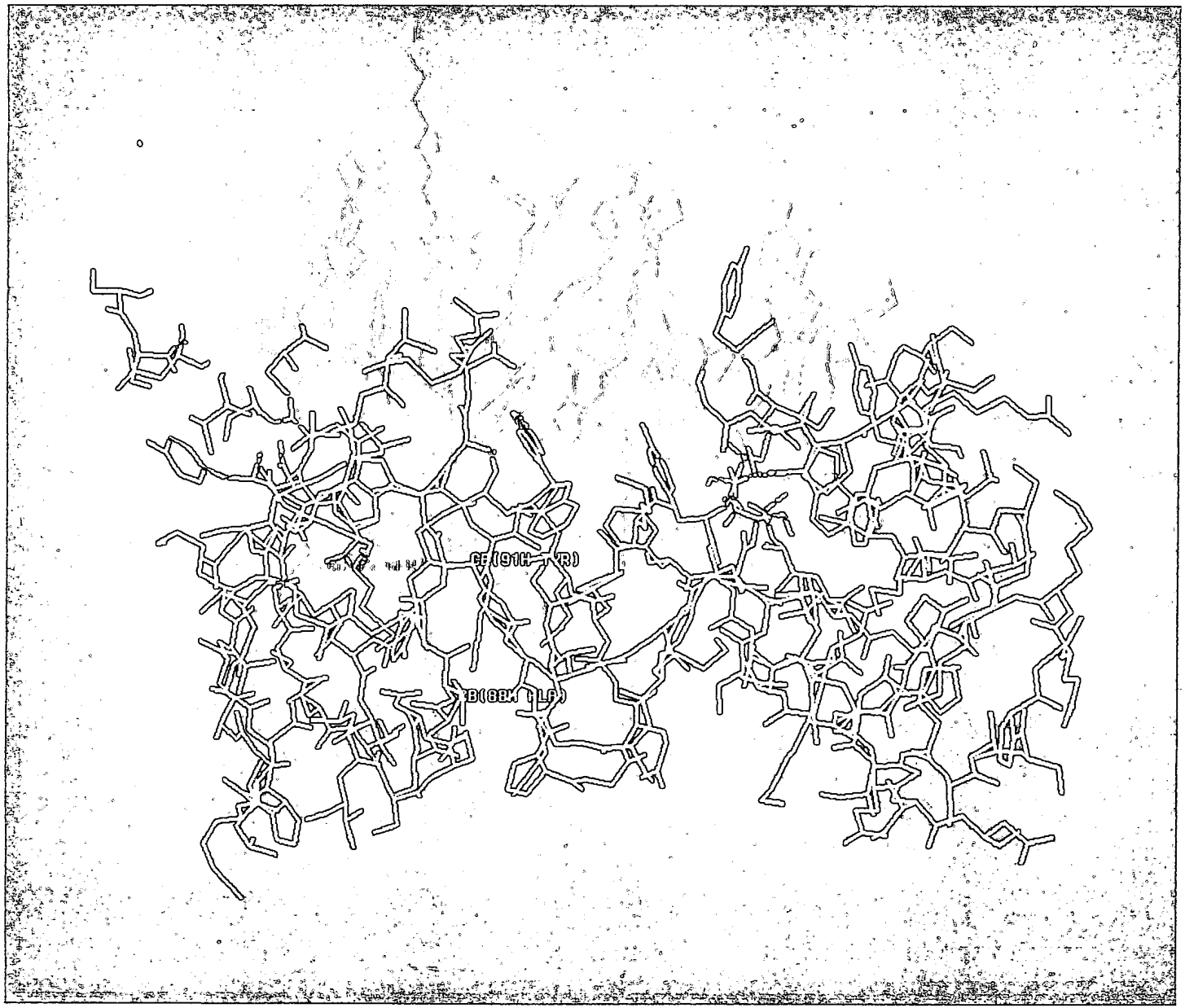


Figure 2A

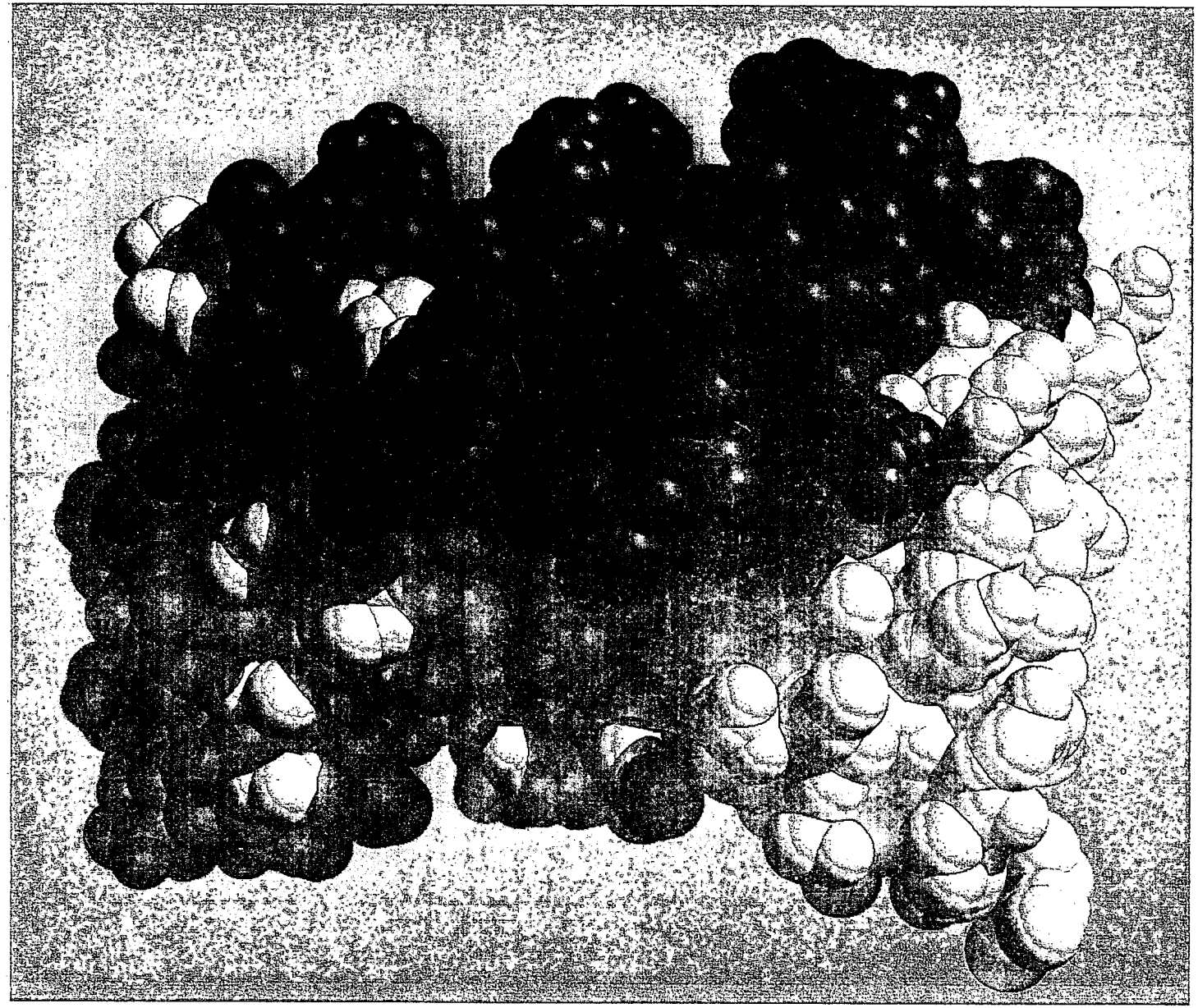


Figure 1A

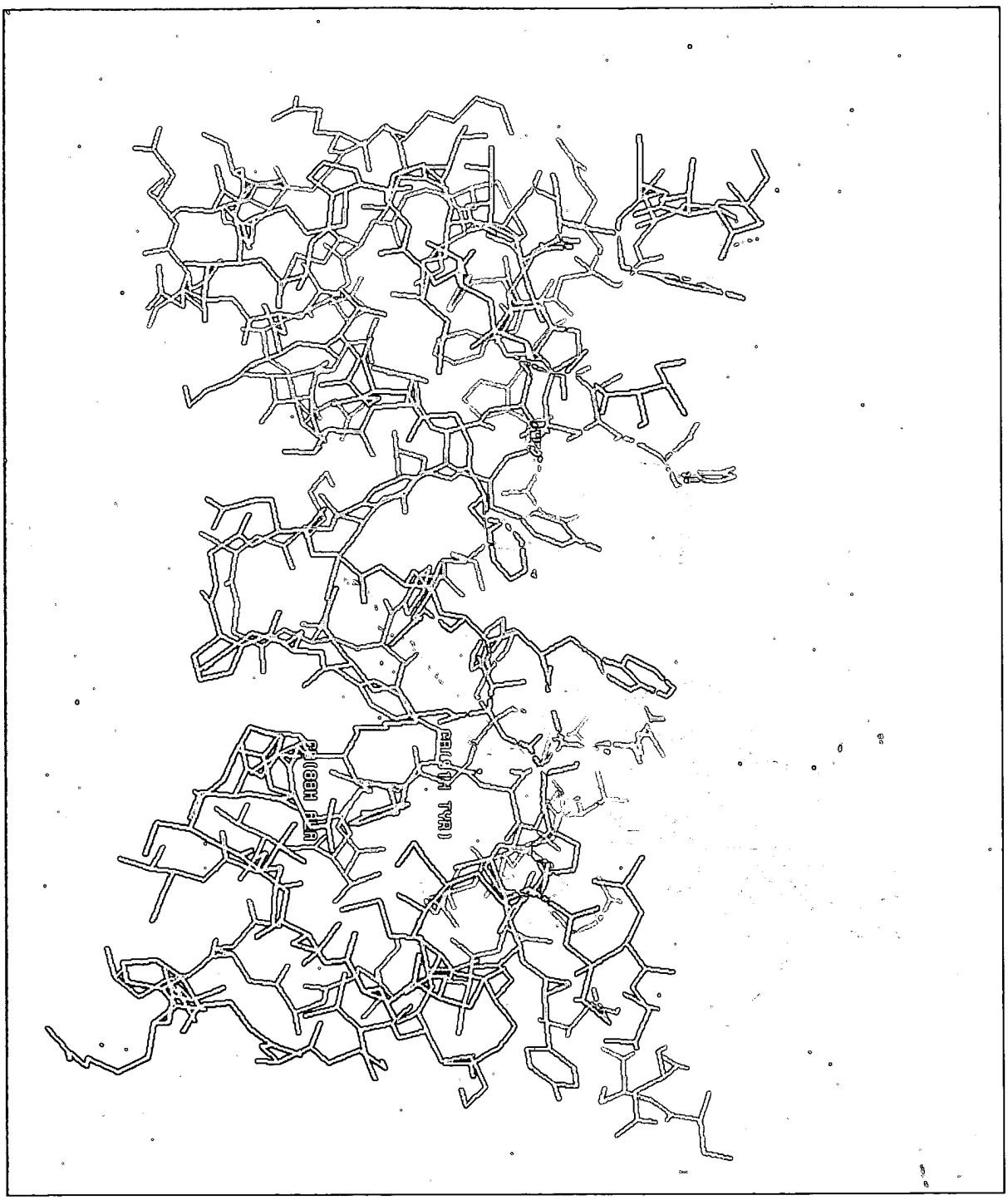


Figure 1B

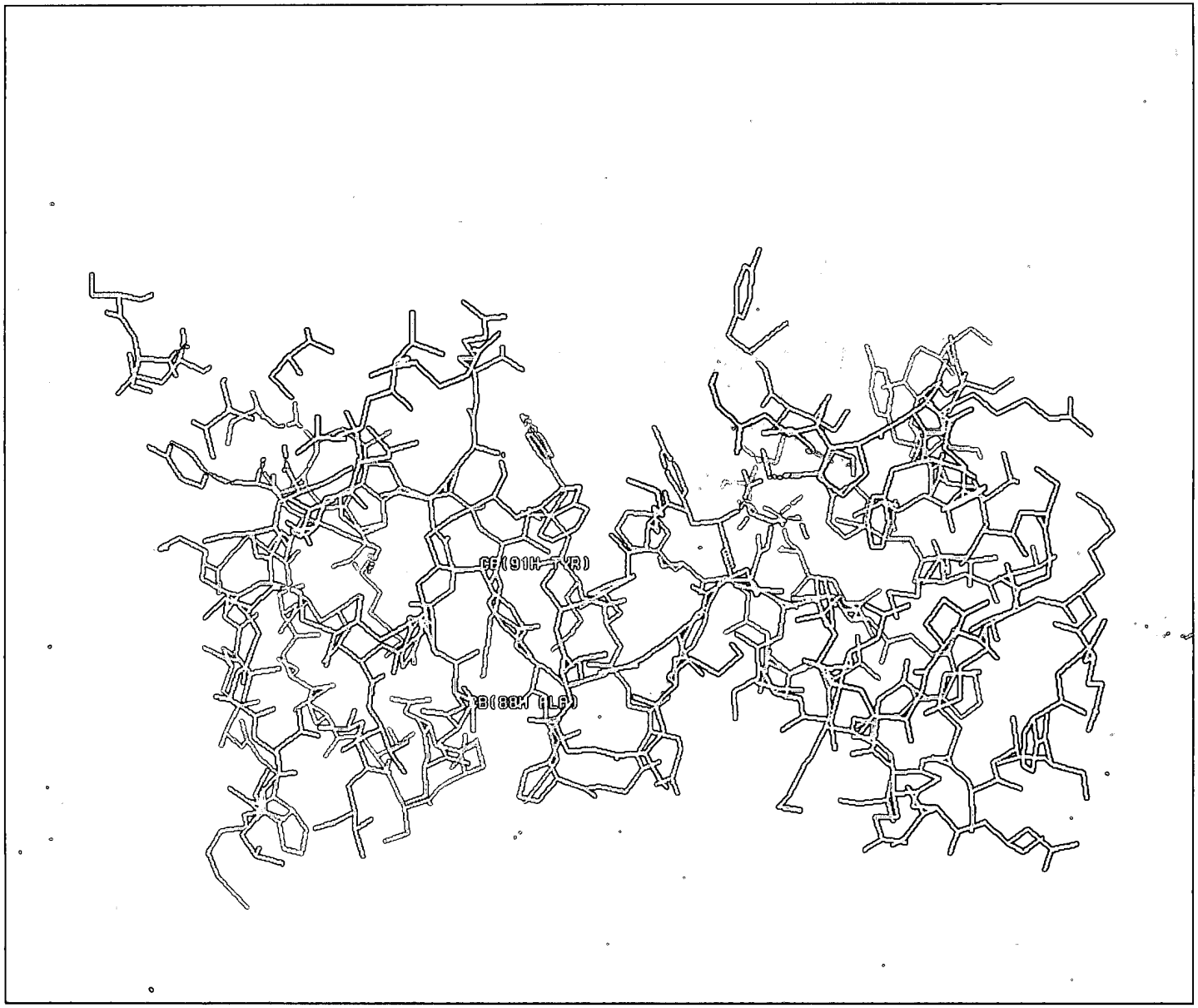
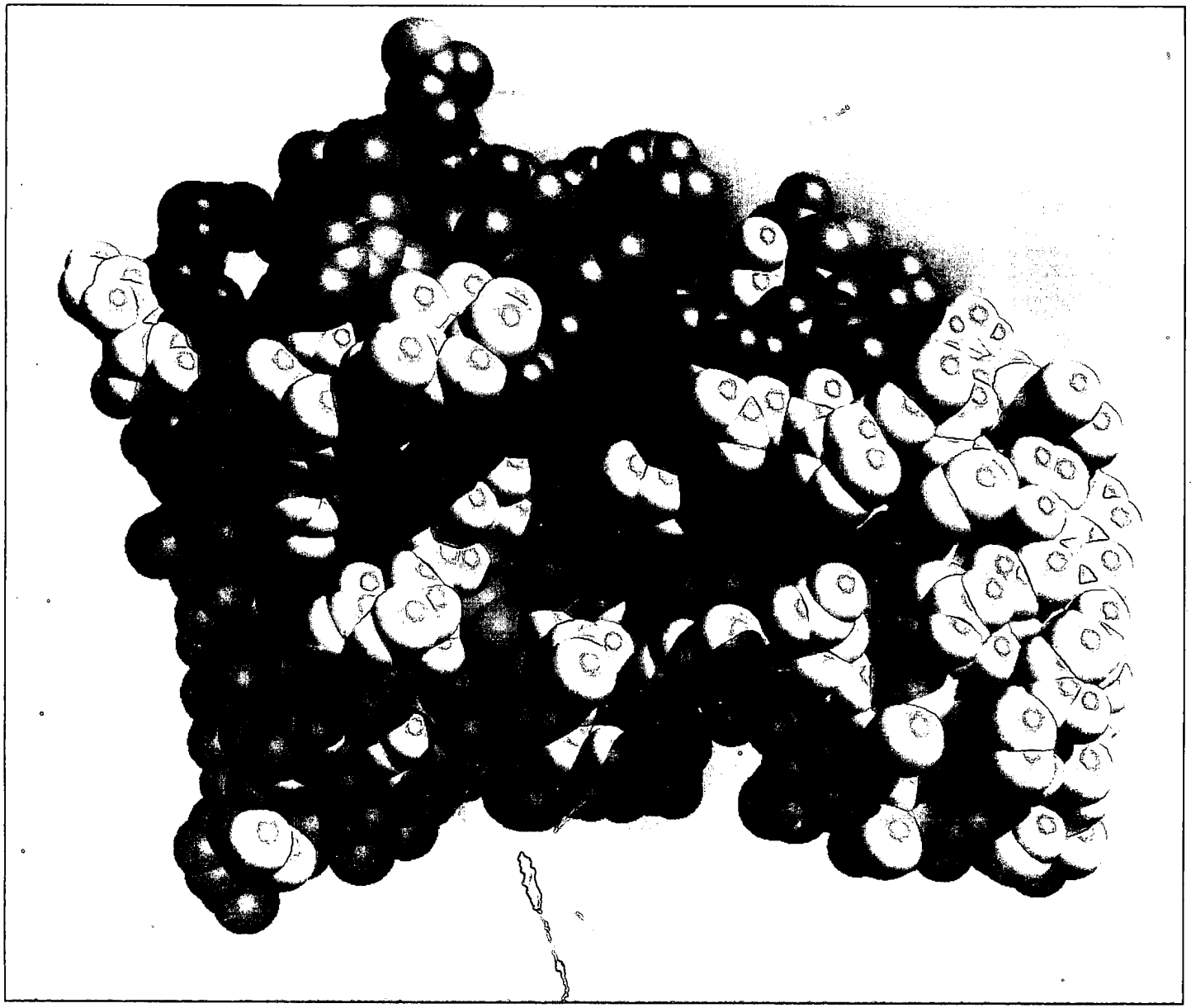
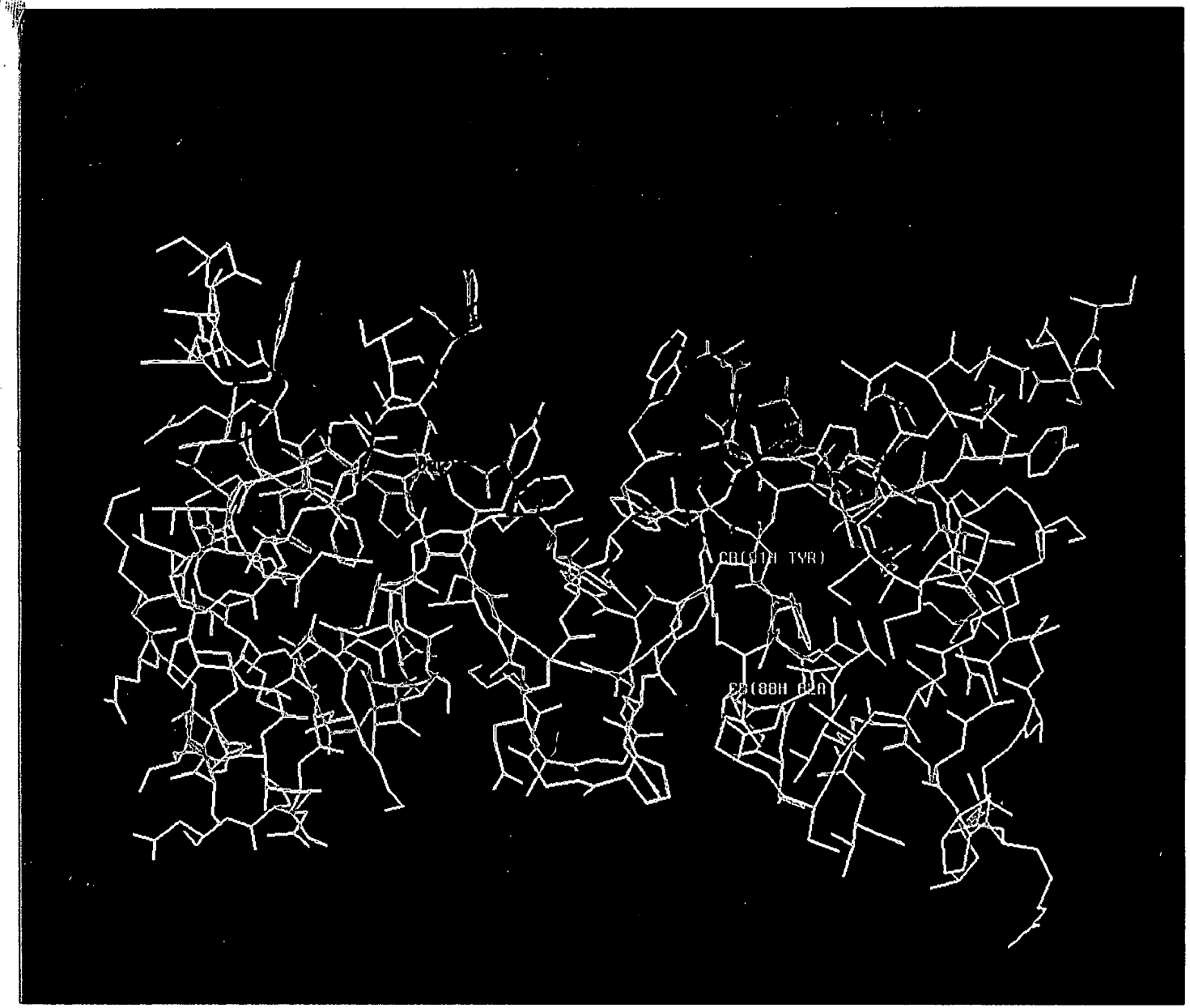


Figure 2A

Figure 2B




CB(31A TYR)
CB(88A ARG)